DATA EVALUATION RECORD

BAS 183 H (Dicamba techn.)
PC Code: 029801
TXR#: 0050539
MRID#: 48081601

4-Week Dietary Immunotoxicity Study in the Rat
OPPTS 870.7800

Prepared for

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TXR#: 0050539

DATA EVALUATION RECORD

STUDY TYPE: 4-Week Dietary Immunotoxicity Study – Rat
OPPTS 870.7800

PC CODE: 029801 **DP BARCODE:** D378374

TEST MATERIAL (PURITY): BAS 183 H (Dicamba techn.) (92.9%, a.i.)

SYNONYMS: 3,6-dichloro-2-methoxy-benzoic acid; Dicamba

CITATION: Buesen, R., V. Strauss, K. Kuttler, et al. (2010) BAS 183 H (Dicamba techn.) – immunotoxicity study in male Wistar rats – administration via the diet for 4 weeks. Experimental Toxicology and Ecology, BASF SE (67056 Ludwigshafen, Germany). Study Identification No. 42S0267/97076, April 21, 2010. MRID 48081601. Unpublished.

SPONSOR: BASF Corporation, P.O. Box 13528, Research Triangle Park, NC 27709-3528.

EXECUTIVE SUMMARY:

In an immunotoxicity study (MRID 48081601), BAS 183 H (Dicamba techn.) (92.9% a.i., Lot No. COD 001266) was administered to 8 male Crl:WI (Han) Wistar rats/dose in the diet at dose levels of 0, 500, 1500, or 4000 ppm (equivalent to 0, 37, 108, or 307 mg/kg/day) for 28 days. The male rat has been determined as the appropriate species/sex for this study. Cyclophosphamide monohydrate in water was administered daily by gavage to the positive control group (8 male rats) at a rate of 4.5 mg/kg/day. On Day 23, animals were immunized with an intraperitoneal injection of 0.5 mL sheep red blood cells (SRBCs) in 0.9% saline (4 x 10^8 SRBCs)/mL). On Day 29, all animals were sacrificed and T-cell dependent antibody responses (TDAR) were evaluated with an enzyme-linked immunosorbent assay (ELISA).

There were no treatment-related effects on clinical signs, mean body weight, mean body weight gain, or mean food and water consumption. In the positive control group, mean body weights were lower than the control value from Day 3 through Day 28, the differences reaching statistical significance ($p<=0.05$) when measured on Days 24 and 28. Body weight gain in the positive control group also was consistently lower than the control group throughout the study, and was statistically significant over most of the measured intervals ($p<=0.05$) within the study, and over the entire study (i.e., Day 0-28, $p<=0.01$). Additionally, food consumption in the positive control

group was lower than the control throughout the study; these data were not statistically analyzed. The decreases in weight, weight gain, and food consumption in the positive control group were considered to be treatment (cyclophosphamide)-related. No unscheduled mortalities occurred in any study group. The NOAEL for systemic toxicity related to treatment with BAS H 183 (dicamba techn.) is 4000 ppm (307 mg/kg/day), the highest dose tested. A LOAEL was not established.

There were no treatment-related changes in anti-SRBC IgM titers as measured by ELISA assay. The mean absolute and relative thymus weights did not differ significantly from the control in any test substance treatment group. In the positive control group, mean anti-SRBC IgM titers were markedly lower than the control, and absolute and relative spleen and thymus weights were significantly reduced when compared with the control ($p<=0.01$).

The Natural Killer (NK) cell activity was not evaluated. Evaluation of toxicity database of dicamba including subchronic, chronic toxicity and reproduction studies showed no treatment-related effects on spleen and thymus weights and histopathology parameters that would suggest the potential for immunotoxicity. Under the HED guidance, if the TDAR assay is negative and evaluation of observational endpoints from all available toxicology database provide no evidence of immunotoxicity, the test article is considered negative for immunotoxicity and evaluation of NK cells activity is not necessary.

Under conditions of this study, the NOAEL for immunotoxicity in male rats is 4000 ppm (307 mg/kg/day), the highest dose tested. A LOAEL was not established.

This 4-week dietary immunotoxicity study in the rat is **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. There were no claims of data confidentiality. The study was conducted in accordance with The OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act), which meet the USEPA GLP standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)], with the exception that recognized differences exist between the GLP Principles/Standards of OECD and those of FIFRA and TSCA.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: BAS 183 H (Dicamba techn.)

Description:	white solid
Lot/batch #:	Batch No.: COD-001266
Purity:	92.9 % a.i. (tolerance ±1%)
Compound stability:	Stable until October 31, 2011 (study code 345682_4); stored at room temperature
CAS # of TGAI:	1918-00-9
Structure:	[HYPERLINK "http://en.wikipedia.org/wiki/File:Dicamba.png"]

2. Vehicle and/or positive control: The vehicle was a ground basal diet (Kliba maintenance diet mouse/rat "GLP", meal). Cyclophosphamide monohydrate (Sigma-Aldrich, Taufkirchen, Germany; Batch ID 1362353, 100% purity; stable until October 2010) was used as the positive control.

3. Test animals:

Species:	Rat (male)
Strain:	Crl:WI (Han)
Age/weight at study initiation:	42±1 days; 174.9 to 200.3 grams
Source:	Charles River Laboratories, Research Models and Services GmbH, Sulzfeld, Germany
Housing:	H-Temp (PSU) cages (TECNIPLAST, Germany), 2065 cm^2 floor area; 4 animals/cage; Lignocel dust-free bedding (SSNIFF, Soest, Germany); wooden gnawing blocks ((Typ NGM E-022) provided (Abedd® Lab. And Vet. Service GmbH, Vienna, Austria); bedding and enrichment regularly assayed for chlorinated hydrocarbon and heavy metal contamination
Diet:	Ground Kliba maintenance diet mouse/rat "GLP", meal (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*; food was withheld for approximately 16-20 hours before necropsy; food was assayed by the supplier for chemical/microbial contamination
Water:	Public drinking water supplied in water bottles, *ad libitum*; water is periodically assayed for chemical contamination by the municipal authorities of Frankenthal and by the Environmental Analytics Water/Steam Monitoring Department of BAF SE, and for microbial contamination by a contract laboratory
Environmental conditions:	**Temperature:** 20 to 24EC **Humidity:** 30 to 70% **Air changes:** 15/hour **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	8 days (interval between day of arrival and first day of test substance administration)

B. STUDY DESIGN:

1. **In life dates:** Start: January 5, 2010; End: February 11, 2010

2. **Selection of test species and gender:** The Wistar rat was chosen as the most sensitive rat species; males were selected because previous studies have shown them to be the more

sensitive sex.

3. **Animal assignment**: Animals were assigned to test groups prior to the first detailed clinical observation on the day preceding the initiation of test substance administration (Day -1). Randomization instructions were compiled with a computer. Animals were assigned to the test groups noted in Table 1. Animals in Group 4 (positive control) were administered cyclophosphamide monohydrate in drinking water by gavage daily for 4 weeks (10 mL/kg body weight) at a dose rate of 4.5 mg/kg/day; the dosage was based on the most recently recorded body weight.

TABLE 1. Study design

Test group	Concentration of BAS 183 H (Dicamba techn.) in the diet (ppm)	Mean group dose (mg/kg/day) [a]	# Animals (males)
Group 0 – Control [b]	0	0	8
Group 1 – Low Dose	500	37	8
Group 2 – Mid Dose	1500	108	8
Group 3 – High Dose	4000	307	8
Group 4 – Positive Control [c]	0	0	8

[a] Data were obtained from page 38 (Section 4.2.6, no table number) of the study report.

[b] Vehicle control (referred to in this review as the "control").

[c] Orally dosed daily by gavage with 4.5 mg/kg cyclophosphamide monohydrate.

3. **Dose selection rationale:** The high-dose dietary concentration of the test substance (4000 ppm BAS 183 H) was selected based on the results of ADME[1], 15-day repeat dose[2], and 90-day repeat dose[3] kinetic studies (the references cited in the report were not available to the reviewer). The approach was said to be consistent with disproportionately high compartment levels in saturation kinetics being a result of systemic toxicity. The combined data from these studies included saturation of excretion pathways, greater systemic exposure and increased half-life, and clinical signs of toxicity reported at approximately 2000 ppm. Therefore, a high dose level of 4000 ppm was considered by the investigators to satisfy, and in some ways, exceed the maximum tolerated dose (MTD) criteria for Wistar rats. Additionally, a 2008 HIARC review[4] concluded that repeat dietary exposures equivalent to 300 mg/kg/day met the

[1] 1) Briswalter, C. (2003) The metabolism of [phenyl-U-^{14}C] SAN 837 H in the rat. Syngenta unpublished report. Syngenta Crop Protection AG, Basel, Switzerland; 2) Hassler (2002) Absorption, distribution, depletion and excretion of 14C-SAN 837 H in the rat. Syngenta unpublished report No. 050AM0. Syngenta Crop Protection AG, Basel, Switzerland.

[2] Leibold, E. et al. (1998) ^{14}C-Dicamba – Study of the plasma kinetics in rats. Syngenta unpublished report No. 02B0266/976009. BASF Toxicology Laboratory, Ludwigshafen, Germany.

[3] Beimborn, D.B. (2003) ^{14}C-Dicamba – Study of the plasma kinetics in rats after repeated oral administration. Syngenta unpublished report No. 02B0439/026013. BASF Experimental Toxicology Laboratory, Ludwigshafen, Germany.

[4] USEPA (2008) Dicamba: Human-health risk assessment for proposed Section 3 new uses on sweet corn. Petition No. 0E6209; PC Code: 029801 DP: 340156 Decision: 304187; Office of Prevention, Pesticides and Toxic Substances, Washington DC 20460.

criteria for MTD. The dose level of cyclophosphamide monohydrate (positive control) was chosen because it was considered to be sufficient to cause immunosuppressive activity.

4. **Diet preparation and analysis:** To achieve the dietary concentrations, the test substance was ground, sieved, weighed out, and mixed with a small amount of food to derive a premix. Depending on the dose group, an appropriate amount of food was added to the premix, and the resulting mixture was blended in a laboratory mixer for approximately 10 minutes. Diets were prepared weekly. Homogeneity and concentration control analyses were performed on 3 dietary samples collected from each of the treatment groups at the beginning of the administration period; two analytical measurements were made for each sample. Samples for the homogeneity and concentration control analyses were stored in a freezer between sampling and analysis (4-5 day interval). The stability of BAS 183 H (Dicamba techn.) at ambient conditions over a period of 10 days was determined using samples from the 500 ppm diet; for this analysis, 4 samples were collected on Day 0 and 3 samples were collected on Day 10. BAS 183 H (Dicamba techn.) concentrations for the stability, homogeneity, and concentration analyses were determined using high performance liquid chromatography (HPLC).

Results:

Homogeneity analysis: The investigators concluded that the test substance was distributed homogeneously in the diet, based on the low standard deviation of the mean percent of nominal concentration calculated for the samples from each test substance group. The mean percent of the nominal concentration (±SD) was 99.4±2.8%, 97.1±3.9%, and 92.4±4.2% for the samples analyzed from the 500, 1500, and 4000 ppm diets, respectively. Although 3 samples were collected from each dietary mixture, it was not indicated in the report from where in the dietary mixture the samples were collected, either relative to the container or to each other.

Concentration analysis: The means of the duplicate analyses of samples collected from each of the test substance treatment groups were within -12% of their respective nominal concentrations. The mean BAS 183 H (Dicamba techn.) concentrations ranged from 487.4 to 513.0 ppm (97.5-102.6% of the nominal concentration) for the 3 samples from the 500 ppm diet, from 1401.3 to 1516.9 ppm (93.4-101.1% of the nominal concentration) for the 3 samples from the 1500 ppm diet, and from 3554.1 to 3879.6 ppm (88.9-97.0% of the nominal concentration) for the 3 samples from the 4000 ppm diet.

Stability analysis: BAS 183 H (Dicamba techn.) was found to be stable in the 500 ppm diet for 10 days at ambient temperature. On Day10, the mean test substance concentration (503 ppm) was 100.8% (±2.9 SD) of the mean content on Day 0 (499 ppm). Concentrations in samples collected on Day 0 ranged from 496 ppm to 506 ppm. Concentrations in samples collected on Day 10 ranged from 490 ppm to 518 ppm.

5. **Immunization:** To assess the acquired (adaptive) immune response of the test animals (T-cell dependent antibody response), on Day 23 animals in all groups (Groups 0-4) received an intraperitoneal dose (0.5 mL injection) of sheep red blood cells (SRBCs) in sterile 0.9% NaCl solution (4 x 10^8 SRBCs/mL).

6. **Statistics:** Mean body weight and body weight change data for Groups 1-3 were compared with the control using Dunnett's test (2-sided) for the hypothesis of equal means and for Group 4 using the t-test (2-sided) for the hypothesis of equal means (the t-test is equal to Dunnett's test in the case of 1 test group). Clinical pathology parameters (i.e., results of the immunotoxicological response) for Groups 1-3 were compared with the control using the Kruskal-Wallis test (non-parametric 1-way analysis). If the resulting p-value was <=0.05, a pair-wise comparison with the control group was performed using the Wilcoxon test for equal medians. Clinical pathology parameters for Group 4 were assessed by a pair-wise comparison with the control group using the Wilcoxon test (2-sided) for equal medians. Organ weight data underwent non-parametric 1-way analysis using the Kruskal-Wallis test (2-sided). If the resulting p value was <=0.05, a pair-wise comparison with the control group was performed using the Wilcoxon test for the hypothesis of equal medians.

   The Reviewer considers the analyses used to be appropriate.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were checked daily for clinical abnormalities. Animals were checked for moribundity and mortality twice daily during the week and once daily on weekends and public holidays.

1b. Clinical examinations: Detailed clinical observations were made in an open field (50 x 37.5 cm, 25 cm high) prior to the start of the administration period and at weekly intervals during the treatment period. The following were assessed: abnormal behavior during handling, fur, skin, posture, salivation, respiration, activity/arousal level, tremors, convulsions, abnormal movements, gait impairment, lacrimation, palpebral closure, exophthalmus, fecal appearance/consistency, urine, and pupil size.

2. **Body weight:** Body weight was determined for animals in all study groups before commencement of test substance administration for randomization of the animals, at the beginning of the administration period (Day 0), twice weekly during administration (Days 3, 7, 10, 14, 17, 21, 24, 28), and on the day of sacrifice (on anesthetized animals). Body weight change was calculated as the difference between the body weight on the day of weighing and on Day 0.

3. **Food/water consumption and compound intake:** Food consumption was determined for each cage in all study groups (Groups 0-4) over a one-day period once weekly (Days 6-7, 13-14, 20-21, 27-28). The average food consumption per cage was used to estimate the mean food consumption/animal/day. Water consumption was assessed by daily visual observation of the water bottles to determine "any overt changes in volume".

   Group mean test substance intakes, expressed as mg/kg/day, were calculated based on individual values for body weight and food consumption. Individual values were determined as follows:

$$\text{Test substance intake for Day x (mg/kg)} = \frac{FC_x \times C}{BW_x}$$

where,

FC_x = mean daily food consumption on Day x (grams)
C = concentration in the food on Day x (mg/kg)
BW_x = body weight on Day x (grams)

4. **Sacrifice and pathology:** On the morning of sacrifice (Day 29), blood was taken from the retro-orbital venous plexus from fasted animals under isoflurane anesthesia; the blood was used for measuring anti-SRBC IgM concentration with ELISA. Animals were sacrificed under isoflurane anesthesia by decapitation. Terminal body weights were determined for the anesthetized animals.

4a. Gross pathology: Animals were assessed by gross pathology. No details of the assessment process were provided.

4b. Organ weights: Spleen and thymus weights were determined for each sacrificed animal.

4c. Organ/tissue fixation: Spleens, thymuses, and gross lesions were fixed in a 4% buffered formaldehyde solution.

5. Immunotoxicity:

a. **Anti-SRBC IgM, ELISA**: The immune response was evaluated using an enzyme-linked immunosorbent assay (ELISA) in accordance with Temple et al. (1995)[5]. The method was modified as follows: a standard curve (8 standards in a 2-fold dilution) was made using an anti-SRBC IgM positive serum pool, for comparison to subsequent test runs (arbitrary lab units/mL: stock standard aliquots stored at –80 °C). Each serum sample was applied to the ELISA in 1:64 and 1:128 dilutions; the 1:128 dilution was generally reported. The ELISA was measured with a Sunrise MTP-reader, Tecan AG, Maennedorf, Switzerland, and evaluated with the Magellan-Software of the instrument manufacturer.

b. **Natural Killer (NK) cell activity assay**: Not performed.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No clinical signs were observed in any test substance-treated group or in the positive control group (Groups 1-4). One animal in the control group (Group 0) had a bilateral skin lesion on the neck from Day 14 to Day 28; the lesion was considered to

[5]Temple L., L Butterworth, T. Kawabata, et al. (1995) ELISA to measure SRBC specific serum IgM: method and data evaluation. In: Methods of Immunology, Volume A, pp. 137-157.

have been spontaneous.

2. **Mortality:** No unscheduled mortalities occurred during the course of the study.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** There were no test substance (BAS 183 H) related effects on mean body weight or body weight gain. During the 4-week treatment period, mean body weights increased in all study groups. In the positive control group, from Day 3 through Day 28, all mean body weights were lower than the control, with statistical significances ($p<=0.05$) on Days 24 and 28 (-4.61% and –4.98% compared with control, respectively). Mean body weight gains for the positive control group also were significantly lower than the control over Days 0-7, 0-14, 0-21, and 0-24 ($p<=0.05$), and over Days 0-28 ($p<=0.01$). Compared with the control group, the percent difference was –11.76, -10.93, -10.12, -12.27, and –12.62% for each of these time periods, respectively. The decreased mean body weights and body weight gains in the positive control group were considered to be treatment (i.e., cyclophosphamide)-related. Mean body weights and mean body weight gains for all study groups during the treatment period are presented in Tables 2 and 3, respectively.

TABLE 2. Average body weight during the treatment period [a]

Treatment group (ppm BAS 183 H)	Mean body weight (grams±SD) (% difference from control)								
	Day 0	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21	Day 24	Day 28
Males (n=8)									
0 (Control)	186.38 ±7.58 (0.00)	204.48 ±8.00 (0.00)	229.31 ±8.60 (0.00)	242.66 ±11.25 (0.00)	2.62.29 ±11.78 (0.00)	274.45 ±12.75 (0.00)	287.26 ±14.21 (0.00)	301.36 ±14.59 (0.00)	310.61 ±14.91 (0.00)
500	187.79 ±5.86 (0.76)	206.03 ±7.34 (0.76)	230.96 ±9.67 (0.72)	247.45 ±9.06 (1.97)	268.09 ±10.38 (2.21)	284.40 ±11.47 (3.63)	297.05 ±11.35 (3.41)	306.57 ±12.48 (1.73)	318.20 ±14.45 (2.44)
1500	187.02 ±8.39 (0.35)	207.75 ±9.40 (1.60)	234.84 ±10.35 (2.41)	249.41 ±12.66 (2.78)	268.31 ±13.93 (2.30)	282.52 ±16.44 (2.94)	295.16 ±18.60 (2.75)	307.16 ±19.07 (1.92)	317.12 ±19.19 (2.10)
4000	188.71 ±5.79 (1.25)	207.90 ±7.18 (1.68)	234.08 ±8.74 (2.06)	251.25 ±10.17 (3.54)	273.07 ±11.24 (4.11)	283.86 ±14.81 (3.43)	300.20 ±14.03 (4.50)	309.18 ±14.79 (2.59)	325.03 ±15.78 (4.64)
Positive Control – cyclophosphamide monohydrate	186.59 ±7.53 (0.11)	202.50 ±6.98 (-0.97)	224.47 ±7.42 (-2.11)	237.28 ±6.55 (-2.22)	252.20 ±7.63 (-3.08)	267.56 ±9.17 (-2.51)	277.26 ±9.04 (-3.48)	287.46* ±9.07 (-4.61)	295.15* ±8.43 (-4.98)

[a] Data were obtained from pages 51 to 56 (Part I, Part A, Tables IA-5 to IA-10) of the study report.
* $p<=0.05$

TABLE 3. Average body weight gain during the treatment period [a]								
Treatment group (ppm BAS 183 H)	Mean body weight gain (grams±SD) (% difference from control)							
	Days 0-3	Days 0-7	Days 0-10	Days 0-14	Days 0-17	Days 0-21	Days 0-24	Days 0-28
Males (n=8)								
0 (Control)	18.10 ±2.53 (0.00)	42.94 ±4.08 (0.00)	56.29 ±6.20 (0.00)	75.91 ±7.74 (0.00)	88.08 ±8.80 (0.00)	100.89 ±11.13 (0.00)	114.99 ±12.14 (0.00)	124.24 ±12.18 (0.00)
500	18.24 ±2.12 (0.76)	43.17 ±4.65 (0.55)	59.66 ±4.57 (6.00)	80.30 ±7.14 (5.78)	96.61 ±8.39 (9.69)	109.26 ±8.20 (8.30)	118.79 ±9.86 (3.30)	130.41 ±11.94 (4.97)
1500	20.72 ±1.60 (14.50)	47.81 ±3.56 (11.35)	62.39 ±6.37 (10.84)	81.29 ±6.96 (7.08)	95.50 ±9.25 (8.43)	108.14 ±11.50 (7.19)	120.14 ±12.06 (4.48)	130.10 ±12.06 (4.72)
4000	19.19 ±2.43 (6.01)	45.36 ±4.86 (5.65)	62.54 ±7.06 (11.10)	84.36 ±8.69 (11.13)	95.15 ±12.29 (8.03)	111.49 ±11.50 (10.51)	120.46 ±12.11 (4.76)	136.31 ±12.79 (9.72)
Positive Control – cyclophosphamide monohydrate	15.91 ±1.87 (-12.09)	37.89* ±2.98 (-11.76)	50.69 ±4.89 (-9.95)	67.61* ±6.07 (-10.93)	80.97 ±5.49 (-8.06)	90.67* ±5.43 (-10.12)	100.88* ±5.98 (-12.27)	108.56** ±6.60 (-12.62)

[a] Data were obtained from pages 57 to 60 (Part I, Part A, Tables IA-11 to IA-14) of the study report.
* Statistically different from the control ($p<=0.05$) using the t-test (= Dunnett's test for 1 test group).
** Statistically different from the control ($p<=0.01$) using the t-test (= Dunnett's test for 1 test group).

C. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Mean food consumption on Day 27-28 was decreased in the 500 and 1500 ppm treatment groups when compared with the control (-1.50% and –5.71%, respectively), but not in the 4000 ppm group (i.e., there was no consistent dose-related trend). The observed decreases in food consumption were not statistically significant and were said to be within the normal range of variation for this strain of rat. In the positive control group, mean food consumption compared to controls was reduced on all days on which it was measured, with a maximum of –9.13% on Day 27-28. The decreases in mean food consumption in this group are consistent with the decreases in mean body weight and body weight gains also observed in the group. The decreases in food consumption in the positive control group were considered to be treatment (cyclophosphamide)-related; food consumption data were not statistically analyzed. The average food consumption for all study groups is presented in Table 4.

TABLE 4. Average food consumption during the treatment period[a]				
Treatment group (ppm BAS 183 H)	Mean food consumption (grams/day) (% difference from control)			
	Day 6-7	Day 13-14	Day 20-21	Day 27-28
Males (n=2 [b])				
0 (Control)	19.75 (0.00)	19.29 (0.00)	19.63 (0.00)	20.65 (0.00)
500	19.76 (0.05)	20.47 (6.14)	20.62 (5.07)	20.34 (-1.50)
1500	20.48 (3.70)	19.31 (0.13)	20.26 (3.21)	19.47 (-5.71)
4000	20.67 (4.66)	20.87 (8.22)	21.68 (10.42)	22.74 (10.12)

Positive Control – cyclophosphamide monohydrate	19.34 (-2.08)	18.73 (-2.90)	19.49 (-0.71)	18.77 (-9.13)

[a] Data were obtained from pages 49 and 50 (Part I, Part A, Tables IA-3 and IA-4) of the study report. These data were not statistically analyzed.
[b] n=number of cages

2. **Test substance consumption:** The average daily doses of BAS 183 H (Dicamba techn.) calculated for Groups 1-3 (3 treatment groups) are presented in Table 5.

TABLE 5. Average dose[a]

Treatment group (ppm BAS 183 H)	Mean achieved dose (mg/kg/day)				
	Day 6-7	Day 13-14	Day 20-21	Day 27-28	Mean
Males					
500	42.72	38.20	34.71	31.93	36.89
1500	130.78	107.98	102.92	92.12	108.45
4000	353.34	305.78	288.79	279.84	306.94

[a] Data were obtained from page 61 (Part I, Part A, Table IA-15) of the study report.

3. **Food efficiency:** Food efficiency was not determined.

4. **Water consumption:** No substance-related findings regarding water consumption were reported.

D. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** No gross lesions were observed in the control or test substance treatment groups. In the positive control group, 6 of 8 animals exhibited macroscopically smaller spleen and thymus sizes. The decrease in organ sizes was consistent with the decreased mean spleen and thymus weights seen in this group and was an expected result.

2. **Histopathology:** A histopathological examination was not carried out.

3. **Organ weights:** Mean terminal body weights, and mean absolute and relative spleen and thymus weights for the test substance treatment groups were not statistically different from the control. Mean absolute and relative spleen weights were slightly lower in the 1500 ppm treatment group (87% and 85% of control, respectively) than the controls. This trend was not dose-related (mean absolute and relative spleen weights in the 4000 ppm group were 99% and 95%, respectively) and was not considered biologically relevant. In the positive control group, absolute and relative spleen and thymus weights decreased significantly compared with the control ($p<=0.01$). In the positive control group, mean absolute and relative spleen weights were 59% and 62% of the control, respectively, and mean absolute and relative thymus weights were 47% and 50%r of the control, respectively. The mean terminal body weight of the positive control group also was significantly lower ($p<=0.05$) than the control. Absolute and relative spleen and thymus weights and terminal body weights are presented for all groups in Table 6.

TABLE 6. Terminal body, spleen and thymus weights[a]					
Treatment group (ppm BAS 183 H)	Mean weight (% of control)				
	Terminal body weight (grams ±SD)	Spleen		Thymus (mg)	
		Absolute weight (grams ±SD)	Relative weight [b] (%)	Absolute weight (mg ±SD)	Relative Weight [b] (%)
Males (n=8)					
0 (Control)	291.188±15.051 (100)	0.6±0.073 (100)	0.206±0.024 (100)	483.75±84.471 (100)	0.166±0.023 (100)
500	298.75±13.072 (103)	0.608±0.069 (101)	0.203±02 (99).	499.0±24.107 (103)	0.167±0.014 (101)
1500	297.963±18.15 (102)	0.524±0.065 (87)	0.176±0.024 (85)	504.5±87.87 (104)	0.169±0.023 (102)
4000	301.188±13.691 (103)	0.593±0.087 (99)	0.196±0.023 (95)	502.25±91.165 (104)	0.166±0.026 (101)
Positive Control – cyclophosphamide monohydrate	276.0*±7.865 (95)	0.351**±0.063 (59)	0.127**±0.023 (62)	228.875** ±97.888 (47)	0.083**±0.036 (50)

[a] Data were obtained from pages 64 to 67 (Part I, Part C, Tables IC1 to IC5) of the study report.

[b] ratio of organ weight to terminal body weight x 100

* Statistically different from the control (p<=0.05) using the Wilcoxon test (2-sided).

** Statistically different from the control (p<=0.01) using the Wilcoxon test (2-sided).

E. IMMUNOTOXICOLOGY:

a. Anti-SRBC IgM, ELISA

The results of the ELISA assay indicated no relevant changes in the anti-SRBC IgM titers in the test substance treatment groups when compared with the control. Mean IgM titers in all treatment group were greater than those of the control, although the median value for the 4000 ppm group 1775 LU/mL) was noticeably lower than the control value (2387 LU/mL). The mean IgM titer for the positive control group was considerably significantly lower than the control (533 vs. 2452 LU/mL). However, the difference was not shown to be statistically significant because of high variability of SRBC IgM titers in the (vehicle) control group, specifically the results for two animals (No. 5 and No.8), both of which had titers below those of the positive control group. When the IgM titers of the positive control group in the present study (mean of 533 LU/mL, median of 491 LU/mL) are compared with historical control data from two clinical pathology tests (means of 740 and 584 LU/mL; medians of 820 and 526 LU/mL), the results of the present study are found to be lower than the historic positive controls. Therefore, the immunosuppression seen in the positive control in the current study was considered by the investigators to be comparable to that observed in previous studies. The results of the ELISA assay are presented in Table 7.

TABLE 7. Results of ELISA assay[a]		
Treatment group (ppm BAS 183 H)	Mean anti-SRBC IgM titer (LU/mL±SD)	Median anti-SRBC IgM titer (LU/mL)
Males (n=8)		
0 (Control)	2452±1809	2387
500	3691±3566	2366
1500	3067±1866	2632
4000	3922±4433	1775

TABLE 7. Results of ELISA assay[a]		
Treatment group (ppm BAS 183 H)	**Mean anti-SRBC IgM titer (LU/mL±SD)**	**Median anti-SRBC IgM titer (LU/mL)**
Males (n=8)		
Positive Control - cyclophosphamide monohydrate	533 [b]±207	491

LU/mL = laboratory unit/milliliter

[a] Data were obtained from pages 62 and 63 (Part I, Part B, Tables IB1 and IB2) of the study report.

[b] Results were considered to be indicative of immunosuppression although statistical significance with respect to the control group could not be demonstrated due to variance in the control group.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigators conclude that, under the study conditions, BAS 183 H (Dicamba techn.) did not produce any signs of immunotoxicity or general systemic toxicity to male Wistar rats when administered in the diet for 4 weeks. The NOAEL was considered to be 4000 ppm (307 mg/kg/day). Administration of cyclophosphamide monohydrate (4.5 mg/kg/day) to a positive control group led to indications of significant immunotoxicity, including lower anti-SRBC IgM antibody titers, and significantly reduced absolute and relative spleen and thymus weights. The results for the positive control group were said to have verified the assay sensitivity. Although statistical significance could not be demonstrated for the IgM data for the positive control group, this was attributed to variance in the data for the (vehicle) control. A comparison of the IgM data for the positive control group in the present study with those of historical positive controls indicates the results are comparable.

B. REVIEWER COMMENTS:

The maximum dose selected for this study (4000 ppm) was based, in part, on the results of prior studies in which clinical signs were said to have been observed at approximately 2000 ppm. In the present study, no clinical signs were reported at 4000 ppm. The choice of the 4000 ppm (307 mg/kg/day) maximum dose level was also based on other types of data (e.g., saturation kinetics). A 2008 HIARC review concluded that repeat dietary exposures equivalent to 300 mg/kg/day met the criteria for MTD. Therefore, the high dose (4000 ppm) in this study is considered acceptable. There were no treatment-related effects on clinical observations, body weight and body weight gain, and food and water consumption. The NOAEL for systemic toxicity is 4000 ppm (307 mg/kg/day), the highest dose tested. A LOAEL was not established.

The investigators attribute the lack of significance of the reduced IgM titer for the positive control group to very low IgM values found for 2 animals in the vehicle control group (267 and 313 LU/mL; data from page 99 of the study report). No explanation is provided for these low values. It is possible that the low response of animals in the control and treatment groups may have been due to SRBCs having been accidentally injected into the intestinal tract, not intraperitoneally. It may be noted that the variability in the range of IgM titers for some of the substance treatment groups was greater than that of the vehicle control. Individual IgM values ranged from 423 to10,370 LU/mL for the 500 ppm group, and from 423 to 13,115

LU/mL for the 4000 ppm group. The standard deviations of the mean for these groups were 3566 and 4433, respectively. By comparison, the range of values for the vehicle control group was 267 to 5330 LU/mL, with a standard deviation of the mean of 1809. Evaluation of the distribution of individual animal data of anti-SRBC titer among the treated and control groups did not show any significant trend indicative of significant suppression of the anti-SRBC response with the treatment of dicamba.

The NK cell activity was not evaluated. Evaluation of toxicity database of dicamba including subchronic, chronic toxicity and reproduction studies showed no treatment-related effects on spleen and thymus weights and histopathology parameters that would suggest the potential for immunotoxicity. Under the HED guidance, if the TDAR assay is negative and evaluation of observational endpoints from all available toxicology database provide no evidence of immunotoxicity, the test article is considered negative for immunotoxicity and evaluation of NK activity is not necessary.

Under conditions of this study, the NOAEL for immunotoxicity in male rats is 4000 ppm (307 mg/kg/day), the highest dose tested. A LOAEL was not established.

C. STUDY DEFICIENCIES:

- There were a few animals with low anti-SRBC IgM responses which were seen among control and treatment groups. No explanation is provided for these low values. It is possible that the low response in the control animals may have been due to SRBCs having been accidentally injected into the intestinal tract, not intraperitoneally. Intravenous injection of SRBC would provide a better response.
- According to the report Summary (page 15 of the study report), Section 1.2 (Observations), lymphocyte counts and determination of lymphocyte subpopulations were performed at the end of the study, in addition to the SRBC IgM antibody titers. No lymphocyte subpopulation data were included in the report. This may have been a misstatement.